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Nitrogen Waste or Nitrogen Source? Urate Degradation in the Renal Sac of Molgulid Tunicates

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Abstract. Two urate-producing ascidians, *Molgula manhattensis* and *M. occidentalis*, were tested for urate oxidase activity. Microradioassays were carried out on the wall and lumen fluid of the urate-containing, molgulid renal sac, on the renal sac endosymbiont *Nephromyces*, and on non-renal sac molgulid tissue. These assays indicate that urate is degraded enzymatically in the renal sac. However, this uricolytic activity is concentrated in *Nephromyces*, rather than in host renal sac tissue.

Thus, the urate "waste" of *Nephromyces*-infected *Molgula* is not stored permanently in the concretions in the renal sac lumen. Since molgulids are universally infected by *Nephromyces* in nature, renal sac function should be reassessed with attention to urate as a nitrogen source as well as a nitrogenous catabolite.

Introduction

The organs of many invertebrate animals have been named before their biological roles were well understood. This is especially true of invertebrate "renal" or "excretory" organs, whose names often reflect surmised, rather than demonstrated, functions (Barrington, 1979). Further, their convergent names mask the diversity of these structures among invertebrates, as well as the dissimilarities of these organs from vertebrate kidneys.

Several invertebrate organs have been assigned an excretory function largely because they contain nitrogenous catabolites which have been identified as excretory products in vertebrate kidneys. If such catabolites are present, need they be destined for excretion? Need they be "waste," whatever the structural, physiological, or ecological context of their production?

Tunicates (Urochordata) possess a number of so-called "renal" tissues (Berrill, 1950; Goodbody, 1974). The functions of these structures are not well understood, despite their functionally evocative names. Because a well-known nitrogenous catabolite, uric acid, has been found in the solid deposits ("concretions") of many of the renal tissues of ascidian tunicates (Azéma, 1928, 1937; Goodbody, 1965; Nolfi, 1970; Sabbadin and Tontodonati, 1967; Saffo, 1977), they often have been assumed to have an excretory function. But many of the features of these tissues seem at odds with such a function. The large renal sac of molgulid ascidians presents a particularly conspicuous puzzle.

First, as a major "excretory" product, uric acid is an unexpected nitrogenous catabolite for these low intertidal or subtidal animals, though evidence (Nolfi, 1970) for an exclusively purine origin for urate in the renal sac of *Molgula manhattensis* may diminish the energetic paradox posed by such a habit.

Second, the renal sac has no ducts or openings at any stage in its development (Saffo, 1978), leading earlier workers (Lacaze-Duthiers, 1874; Das, 1948) to the notion that the renal sac "excretes" urate waste by storing it rather than eliminating it.

Finally, a symbiotic fungus-like protist, *Nephromyces*, inhabits the renal sac lumen of all adult individuals of at least seven molgulid species (6 species of *Molgula*, 1 of *Bostrichobranchus:* Saffo, 1982, and unpub. data on *M. robusta*); earlier observations (Giard, 1888; Buehner, 1930, 1965) cite its presence as well in the renal sac of many other molgulid species. *Nephromyces* is usually present in large numbers in its hosts, especially in adults, with greatest densities typically in the immediate vicinity of the urate-containing, renal sac concretions.

Symbiotic (including parasitic) infection is unusual for excretory organs generally (Hoehberg, 1982), and it remains undetected among "renal" tissues of non-molgulid ascidian families (Saffo, unpub. obs. on Ascidiidae and Corellidae).

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Symbiotic infection is frequently associated with *extrarenal* urate deposits among invertebrates (Buchner, 1965). The guts of termites (Potrikus and Breznak, 1980, 1981; Breznak, 1982) and possibly anobiid beetles (Jurzitza, 1979), the fat body of cockroaches (Mullins and Cochran, 1975a, b; Downer, 1982; Mullins, 1982), the parenchyma of the acoel flatworm *Convoluta* (Douglas, 1983), and the "concrement gland" of terrestrial prosobranch snails (Meyer 1925) all harbor endosymbionts in association with urate deposits. For each of these cases, it has been either suggested or shown that endosymbionts utilize the urate deposits of the host. Even without the involvement of symbionts, extrarenal urate might be neither permanently deposited nor excretory in function (Dresel and Moyle, 1950; Berridge, 1965; Duerr 1967, 1968; Gifford, 1968; Mullins and Cochran, 1975 a, b; Cochran, 1979; Buckner, 1982; Wolcott and Wolcott, 1984). Might *Nephromyces* also use the urate deposits of its host? Might urate be only a transient deposit in *Molgula*?

Goodbody (1965) searched for uricase activity in several ascidians. Using spectrophotometric assays, he found evidence for uricase in only a single ascidian, the styelid *Polycarpa obtecta*, despite examination of a number of ascidians, including *Molgula manhattensis*. However, Goodbody noted "the difficulties of proving a negative result," a problem underscored by the limited sensitivity of spectrophotometric methods for assay of uricase, and of the inclusiveness ("whole animals and portions of the tissues") of his samples.

Using more sensitive radioisotope methods and finer sample partitioning, I have re-investigated the possibility of urate degradation in *Molgula manhattensis* and *Molgula occidentalis*. I have assayed for urate oxidase activity in non-renal sac tissue of *Molgula*, and in both host (*Molgula*) and symbiont (*Nephromyces*) components of the renal sac wall and lumen.

Materials and Methods

Samples were assayed for urate oxidase (uricase; urate: oxygen oxidoreductase, E.C.1.7.3.3) activity with radioisotope methods modified from Friedman *et al.* (Friedman and Merril, 1973; Friedman and Johnson, 1977; Friedman *et al.*, 1985).

Assay method A provided preliminary estimates of uricolytic activity, (Table I) for later comparison with the definitive data (Table II) provided by method B. Method A was also used to compare the R_f values of degradation products of tissue samples with those of commercial urate oxidase. Individual tissue aliquots were incubated 20 to 40 minutes at room temperature (20–22°C) with 2-^{14}C-uric acid (Amersham, 50–51 mCi/mmole) dissolved in 0.03 *M* sodium borate buffer (pH 9.4; Truscoe, 1968). Reaction mixtures consisted of: 1 volume (3–10 μl) sample (buffer, enzyme, cell-free fluid or homogenized cell suspension); 1 volume 0.0010, 0.0012, or 0.0020 *M* ^{14}C-urate in pH 9.4 borate buffer; 2 volumes double-deionized water. Successive aliquots of the reaction mixture were spotted onto a cellulosic thinlayer chromatographic plate, which was developed for 90–120 minutes (to a distance of 14–17 cm) in 0.15 *M* NaCl: 95% ethanol (4:1) (Friedman and Merril, 1973). Each lane of the chromatogram was cut into 1 cm segments, which were assayed for the distribution of [^{14}C] with a liquid scintillation counter.

Assay method B was similar in general design to method A, except for modifications designed to minimize reagent and sample impurities, to maximize enzyme yield from tissue extracts, to standardize assay conditions, and to enable calculation of specific activities. ^{14}C-uric acid (Amersham; 51 mCi/mole) was purified chromatographically before assay to minimize the amount of ^{14}C-allantoin initially present in the urate reagent. Aliquots of the purified urate were resuspended in ultrapure (Milli-Q) water, titrated to pH 9.4 with NaOH, and kept at −70°C until use. Tissues were homogenized at 0°C in a pH 9.1 buffer containing 11.0 m*M* borate, 1.4 m*M* EDTA, and 0.18% Triton X-100 made up with Milli-Q water. To assess specific activity of urate oxidase, a small (2–10) μl aliquot of each tissue homogenate was analyzed for protein content using the method of Bradford (1976; Pierce Chemical Company). The major (15 μl) portion of tissue homogenate was added to 5.9×10^{-6} moles (10–18 μl) of purified ^{14}C-urate, and the resulting reaction mixture incubated at 26°C. Formation of ^{14}C-allantoin (or other reaction products) in these mixtures, and degradation of ^{14}C-urate, were followed for 20 to 40 minutes, using chromatographic methods described for method A.

Enzyme activity detected by method B was calculated from the greatest reaction rate (usually, but not always, the initial rate) observed during the first twenty minutes of incubation. Two measures of activity were calculated: μmoles reaction product/mg prot/min (specific activity) and μmoles reaction product/15 μl volume/min. Though specific activity was the preferred means of expressing activity, activity/volume/min was also included to better compare the significance of enzyme activity in two different sorts of samples: largely acellular fluid (centrifuged renal sac fluid) and tissue homogenates.

Two field-collected molgulid species were assayed for uricolytic activity: *Molgula occidentalis* from Alligator Harbor, on the Gulf Coast of Florida (Gulf Specimen Co., Panacea, Florida), and *Molgula manhattensis* from Woods Hole, MA; Stone Harbor, NJ; and San Francisco Bay, CA. As expected from earlier work (Saffo, 1982), all these field-grown *Molgula* individuals were infected with *Nephromyces*. Animals were maintained in running sea-

water or aerated aquaria and used in assays within a week after collection.

Parallel, laboratory-grown populations of *Nephromyces*-infected and *Nephromyces*-free *Molgula manhattensis* were also assayed to assess directly the impact of *Nephromyces* on urate metabolism in *Molgula*. These lab animals were raised in 0.5 μm-filtered seawater, using methods described previously (Saffo and Davis, 1982).

Several tissue types were analyzed. Each tissue was freshly dissected, or dissected and then frozen at −20°C for one hour and thawed at room temperature, just before use. Single tissue types from one to several (2–11 see Table II) animals were pooled for enzymatic assay. For each laboratory-raised animal, an aliquot of uncentrifuged renal sac fluid was examined by phase contrast microscopy at 400× to confirm the presence or absence of *Nephromyces* from *Nephromyces*-inoculated and uninoculated populations, respectively.

Renal sac samples were of four sorts: (1) fluid from the renal sac lumen, centrifuged 10 (method A) or 15 (method B) minutes at 1000 × *g* to remove suspended cells (which account for a substantial fraction of renal sac fluid volume in *Nephromyces*-infected *Molgula*), (2) *Nephromyces*, removed manually in *M. occidentalis* or collected as a pellet from centrifuged renal sac fluid in symbiont-infected *M. manhattensis*, (3) combined *Nephromyces*-fluid samples, using uncentrifuged renal sac fluid from infected *Molgula* (4) renal sac wall (host tissue: the layer of molgulid cells bounding the renal sac lumen, along with surrounding heart tissue) rinsed in sterile seawater or borate buffer to remove as much renal sac fluid and *Nephromyces* residue as possible and then homogenized in buffer.

Because of the minute volume of each sample, most samples could be assayed only once. Thus, each of the enzyme assays of a particular sort of tissue was carried out on material isolated from a different animal source.

Four other samples, serving as comparative controls, were spotted onto TLC plates and assayed for ^{14}C, with the first three followed over a time course similar to that of the tissue samples. (1) The neural gland complex and adjacent mantle tissue of *Molgula* provided molgulid, non-renal sac tissue. (2) A 6 mg/ml suspension of urate oxidase (Sigma Chemical Co., Type V, porcine liver: about 20 activity units/g protein) in borate buffer compared activity of purified urate oxidase with that of tissue samples. (3) ^{14}C-urate in borate buffer identified the extent of endogenous ^{14}C-allantoin impurity in the labeled uric acid substrate, as well as the rates of spontaneous, non-enzymatic degradation of ^{14}C-urate at alkaline pH (Friedman and Merril, 1973; Antia and Landymore, 1974). (4) Parallel TLC separations of unlabeled 0.01 *M* urate, 0.02 *M* allantoin, and 0.02 *M* urea, dissolved in borate buffer, assisted chromatographic localization of substrate and putative degradation products and provided non-isotopic controls for scintillation counting. Unlabeled uric acid was detected by absorption in ultraviolet light; unlabeled allantoin and urea were detected by the production of yellow color with Ehrlich reagent (4-Dimethylaminobenzaldehyde HCl: Sigma Chemical).

Results

Tables I and II provide clear evidence of enzyme-catalyzed uricolysis in *Nephromyces*-infected *Molgula occidentalis* and *Molgula manhattensis*. This activity is not distributed evenly throughout all molgulid tissues, or even throughout the entire renal sac. Rather, it is concentrated in *Nephromyces* cells.

Identity of uricolytic products

With an NaCl-95% ethanol solvent, the R_f of the peak of uricolytic product (Fig. 1) in the renal sac was quite similar to that of urate oxidase controls. Over two qualitative assays (Method A) and all time points (0, 3, 10, 20, 40 minutes), the urate oxidase-catalyzed reaction product (presumptive allantoin) showed a single [^{14}C] peak at R_f values of .82–.87. In 13 parallel assays, the [^{14}C] maximum of the *Nephromyces*-catalyzed reaction product peak showed an R_f value of 0.79–.87. R_f values for urate oxidase-catalyzed product in quantitative assays (Method B) ranged from .78–.81; those for *Nephromyces*-catalyzed product peak ranged from .75–.80.

In Method A, the R_f of ^{14}C-urate in borate buffer ranged from .43–.45. That of ^{14}C-urate incubated with tissue homogenates ranged from .40–.45. In Method B, the R_f of ^{14}C-urate in borate buffer was .41–.43. That of ^{14}C-urate incubated with tissue homogenates was .41–.44.

TLC separations of nonisotopic controls yielded R_f values of: 0.45–0.49 (urate), 0.80–.84 (allantoin), and 0.86–.90 (urea). TLC separations of unlabeled renal sac fluid yielded numerous UV-absorbent and UV-fluorescent compounds, of a wide range of R_f values. Though some of these compounds (those spanning R_f ranges of .75–.87) overlapped allantoin and urea in mobility, they did not react with Ehrlich's reagent.

Localization of uricolytic activity: assay method A (preliminary assays)

Results from method A were more variable than those from method B. Major complicating factors included variability in solubilization rates of the ^{14}C-urate substrate, and variability in effectiveness of physical methods for tissue homogenization from sample to sample. Nevertheless, enzymatic activity detected by these assays

Table I

Urate oxidase activity — Method A

Enzyme source	# Assays	Δ Product-cpm/minute/sample (standard error)	% Individual assays showing uricolytic activity[a]
Controls			
Buffer	9	56 (±63)	0
Urate oxidase (porcine liver)	2	2,355 (±455)	100
Uninfected *Molgula manhattensis* (lab[c])			
Non renal sac	2	0	0
Renal sac wall	2	0	0
Renal sac fluid # 1	1	0	0
Renal sac fluid #2	1	156	—
Infected *Molgula manhattensis* (field[b] and lab[c])			
Non renal sac (field)	1	0	0
Non renal sac	5	0	0
Renal sac wall (field)	1	0.2	0
Renal sac wall	4	122 (±167)	50
Renal sac fluid (field)	1	34	0
Renal sac fluid #1	2	354 (±78)	100
Renal sac fluid #2	2	2,518 (±1036)	100
Nephromyces (field)	2	1,585 (±1648)	100
Nephromyces #1	2	576 (±517)	100
Nephromyces #2	2	25,894 (±7422)	100
Nephromyces (with fluid) (field)	4	989 (±540)	100
Nephromyces (with fluid)	2	2,110 (±1115)	100
Infected *Molgula occidentalis* (field)[b]			
Renal sac wall	1	0	0
Renal sac fluid	1	539	100
Nephromyces	1	772	100
Nephromyces (with fluid)	1	5,022	100

[a] % Individual assays with Δ product-cpm/min > parallel buffer control.

[b] Field-collected animals.

[c] Laboratory-raised animals.

indicated that urate oxidase activity was concentrated in *Nephromyces*.

Among samples from *Nephromyces*-infected *M. manhattensis* and *M. occidentalis*, all samples of *Nephromyces* cells showed high uricolytic activity. Uricolytic activity was also detected in five of six renal sac fluid samples, though activity per sample volume was always lower than that of parallel *Nephromyces* samples. Of host tissue, only two of six samples of renal sac wall, and no sample of nonrenal sac tissue, showed uricolytic activity.

In general, tissue samples from uninfected *M. manhattensis* showed no uricolytic activity. In the single possible exception to this pattern, one of the two uninfected renal sac fluid samples (renal sac fluid #2, Table I) assayed showed a rise in ^{14}C-product levels 1.2 times greater than that of the parallel buffer control, but only 0.75% and 7.7% that of parallel *Nephromyces* samples (*Nephromyces* #2) and of urate oxidase controls, respectively. Further, levels of ^{14}C-urate substrate fluctuated throughout incubation. Thus, the evidence for urate oxidase activity is ambiguous in this apparently aberrant assay.

Assay method B

Of seven samples of non-renal sac tissue, renal sac wall, and renal sac fluid assayed from uninfected *M. manhattensis* (see Table I), six showed no uricolytic activity. The seventh (one of two renal sac wall samples) showed miniscule urate oxidase activity: an increase in ^{14}C-product of only 60 dpm after 20 minutes' incubation, or about 10^{-3} the specific activity of commercially purified urate oxidase.

Even *Nephromyces*-infected *M. manhattensis* showed no consistent evidence for urate oxidase activity in host tissue. No urate oxidase activity at all was detected in non-renal sac tissues of infected *M. manhattensis*. Three samples of renal sac wall also showed no uricolytic activity, while one showed low activity (10^{-2} that of the urate oxidase control). Two samples of renal sac fluid showed no uricolytic activity, one showed low activity, and a fourth sample showed low uricolytic activity per sample volume, but high specific activity.

In contrast to the absent or spotty urate oxidase activity detected in *Molgula* tissue, all *Nephromyces* samples from *M. manhattensis* showed substantial urate oxidase activity, comparable to or even exceeding the specific activity of commercial urate oxidase.

Distribution patterns of urate oxidase activity in *Nephromyces*-infected *M. occidentalis* resembled those found in *Nephromyces*-infected *M. manhattensis*. Urate oxidase activity was absent in non-renal sac tissue and in one sample of renal sac wall. A second sample of renal sac wall, with small *Nephromyces* clumps adhering after rinsing, showed low urate oxidase activity, about a tenth that of commercial urate oxidase controls. Both samples of renal sac fluid showed relatively low urate oxidase activity per sample volume, but very high inferred or calculated specific activities. All *Nephromyces* samples showed very high specific activities for urate oxidase, either comparable to, or even far exceeding, those of the urate oxidase control.

Urate oxidase activity varied among *Nephromyces* samples from different sources of *Molgula*. The specific activities of urate oxidase measured from field-collected *M. manhattensis* (713–1567 μmoles/min/mg protein) were less than those from either laboratory-raised *M. manhattensis* (4800–6245 μmoles/min/mg) or *M. occidentalis* (4,103–23,696 μmoles/min/mg).

Table II

Urate oxidase activity: assay method B

Enzyme source	# Animals pooled per assay	# Assays (n)	Mean μmoles/min × 10^8 (standard error)	Specific activity: mean μmoles/mg prot/min × 10^5 (standard error)	% Assays with uricolytic activity[a]
Controls					
Buffer	—	3	0	0	0
Urate oxidase (porcine liver)					
0–2 mins	—	1	12,950	4,788	100
2–20 mins	—	1	1,310	490	100
Uninfected *Molgula manhattensis* (lab[c])					
Non renal sac	3–4	2	0 (0)	0 (0)	0
Renal sac fluid	5	2	1.35 (±1.35)	0.55 (±0.55)	0
Renal sac fluid	3–6	3	0 (0)	0 (0)	0
Infected *Molgula manhattensis* (field[b] and lab[c])					
Non renal sac (field and lab)	2–4	3	0 (0)	0 (0)	0
Renal sac wall					
field	2	2	0 (0)	0 (0)	0
lab	4	2	17 (±17)	15 (±15)	50
Renal sac fluid					
field	3	2	9 (±9)	18.5 (±18.5)	50
lab	4–11	2	76 (±76)	2,235 (±2235)	50
Nephromyces					
field	3	2	2,655 (±1761)	1,140 (±604)	100
lab	4–11	3	3,909 (±4256)	5,411 (±748)	100
Molgula occidentalis (field[b])					
Non renal sac	2	1	0	0	0
Renal sac wall[d]	1	2	115 (±115)	115 (±115)	50[d]
Renal sac fluid	1	2	511 (±485)	34,160+[e]	100
Nephromyces	1	3	7,518 (±3212)	14,116 (±9803)	100

[a] % of individual assays showing specific activity >0.
[b] Field-collected animals.
[c] Laboratory-raised animals.
[d] Clumps of *Nephromyces* adhering to wall in the sample showing uricolytic activity.
[e] Protein not detectable in one sample.

Discussion

The data presented in this report indicate that enzymatically catalyzed uricolysis takes place within the renal sac of *Molgula*. They further suggest that uricolytic activity is localized virtually exclusively within the renal sac symbiont, *Nephromyces*, rather than in host tissue. Indeed, the spotty presence of uricolytic activity in *Nephromyces*-infected molgulid tissue, coupled with the general absence of such activity in symbiont-free molgulid tissues, suggests that uricolytic activity detected in host tissue is not endogenous to *Molgula*, but rather arises largely or exclusively from *Nephromyces* contaminants.

Among symbiont-free molgulid tissue samples assayed by method B, no tissues except a single sample of renal sac wall showed any trace of urate oxidase activity. By comparison with urate oxidase controls, with *Nephromyces*, and with other organisms, the activity of even this sample is essentially zero: its value is smaller even than the urate oxidase activity detected in liver and kidney tissue of uricotelic animals (Saffo, 1989: in prep.; Scott *et al.*, 1969).

The low uricolytic activity detected in a small fraction of renal sac wall samples from infected *Molgula* seems best explained as the result of *Nephromyces* remnants occasionally remaining (in one case, visibly so) on the renal sac wall surface after rinsing.

By similar reasoning, uricolytic activity detected in several samples of renal sac fluid of infected animals must result, in some sense, from contamination by *Ne-*

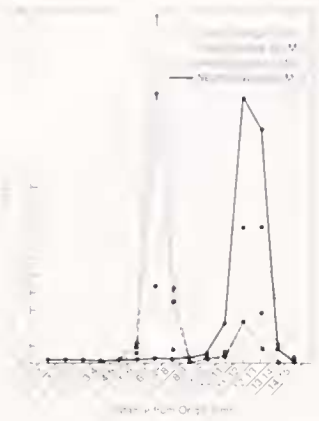

Figure 1. Incubation of ^{14}C urate with *Nephromyces*-renal sac fluid homogenate from field-collected *Molgula occidentalis*, compared with ^{14}C-urate incubated with commercial urate oxidase (assay method A). Distribution of radioactivity (measured as cpm) on thinlayer chromatogram after 0 and 20 minutes' incubation. Urate peak is 6–7 cm from origin; degradation product peak (allantoin) is 11–13 cm from origin.

phromyces. *Nephromyces* could give rise to uricolytic activity in the renal sac fluid in three possible ways. First, the symbiont might induce *Molgula* to produce uricolytic enzymes and to secrete them from the renal sac wall into the renal sac lumen. Since most renal sac wall samples lack detectable enzymatic uricolytic activity, this possibility seems unlikely. A second possibility is that the funguslike trophic stages of *Nephromyces* secrete degradative enzymes extracellularly, into the renal sac fluid, or that enzymatic contents of *Nephromyces* cells might be released naturally into the renal sac fluid as individual symbiont cells die. This possibility does not explain, however, the *absence* of urate oxidase activity from several samples of renal sac fluid drawn from infected *M. manhattensis*. Third, uricolysis in renal sac fluid could be an artifact of centrifugation: that is, the supernatant fluid could still contain intact cells of *Nephromyces* after centrifugation, or the *Nephromyces* cells could have lysed during centrifugation. The two nonuricolytic renal sac fluid samples from infected *M. manhattensis* would thus represent the only samples of renal sac fluid free of cells or cell contents. The discrepancy in these fluid samples, between uricolytic activity per volume (always low) and specific activity (much higher), is further suggestive of contamination of these samples with a small amount of protein.

The similarities between R_f values of urate degradation products resulting from catalysis by commercial urate oxidase and those catalyzed by *Nephromyces* are consistent with the notion that *Nephromyces* produces allantoin from urate—that is, that urate degradation in *Nephromyces* is catalyzed by urate oxidase. This need not mean that allantoin is the final product of urate degradation in *Nephromyces*. The apparent absence of allantoin from HPLC separations of renal sac fluid (Saffo, unpub.) underscores the possibility that allantoin may be only a transitional product of urate degradation in the renal sac.

The chromatographic data also leave open the possibility that additional compounds are produced from urate degradation. The NaCl-95% ethanol TLC solvent, used here because it yields widely different R_f values for urate and allantoin, does not separate allantoin distinctly from other, more polar compounds. For example, urea is only slightly more mobile than allantoin, yielding closely abutting allantoin and urea spots in TLC separations of allantoin-urea mixtures. Further, several compounds in the renal sac fluid overlap allantoin in mobility. Thus, the ^{14}C products of urate degradation seen in *Nephromyces* extracts might include urea or other compounds, in addition to allantoin.

Whatever may be the particular pathways of urate degradation in the renal sac, the general lesson remains clear. In *Nephromyces*-infected *Molgula*—that is, in *Molgula* in nature—urate is *not* a permanent deposit in the renal sac. Thus, uric acid cannot serve as a *permanent* repository for waste nitrogen in the renal sac. The role of urate production and of renal sac function in *Molgula* must be re-examined.

The high rates of uricolysis in *Nephromyces* and the symbiont's high densities in adult molgulids suggest that *Nephromyces* makes an important contribution to the function of the renal sac. Because of *Nephromyces'* widespread, probably universal, infection rate among adult *Molgula*, the metabolic activities of the symbiont and their specific effects on the physiology of *Molgula* merit careful scrutiny.

The present data suggest one aspect of *Nephromyces'* metabolism that is worth particular attention. For *Nephromyces*, renal sac urate is not nitrogen waste, but rather a potential *source* of carbon and nitrogen. Especially if the *Nephromyces*-molgulid symbiosis benefits the molgulid hosts (Saffo, 1984, 1986), could urate serve ultimately (albeit indirectly) as a nitrogen source for *Molgula*, as well as for *Nephromyces*? I am currently pursuing such views, tracing the fate of *Nephromyces* metabolites in and beyond the renal sac, determining whether carbon and nitrogen from urate degradation by *Nephromyces* is reincorporated by *Molgula*, and investigating the long-term consequences of *Nephromyces'* activities for the general biology of *Molgula*.

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